Project Report

# Summary

This project investigated the potential of poly(lactic-co-glycolic acid) (PLGA) microspheres to enhance the photostability of the UV absorber avobenzone. Initial spectrophotometric analyses confirmed avobenzone's high susceptibility to UV radiation and established its maximum absorption peak at 357 nm, distinct from the more stable UV absorber octocrylene, which peaked at 310 nm.

A series of avobenzone-loaded PLGA microsphere formulations (AV9 through AV16) were prepared using PLGA concentrations ranging from 5% to 30%. These formulations achieved drug loading efficiencies between approximately 41% and 49%. Subsequent UV exposure studies demonstrated that encapsulation within PLGA microspheres conferred a protective effect against avobenzone degradation. Formulations with 10% PLGA (AV15) and 20% PLGA (AV12) proved most effective, showing superior protection against UV-induced degradation compared to other polymer concentrations.

The functionality of the microspheres as a delivery system was confirmed in a 3-day drug release study. Selected formulations, AV10 and AV15, exhibited successful release profiles, achieving cumulative drug releases of 113% and 74%, respectively, after 56 hours. These findings indicate that PLGA microspheres are a viable vehicle for stabilizing avobenzone. Future research will focus on the continued synthesis and analysis of the most promising formulations, AV12 and AV15, utilizing more consistent and intense UV radiation sources to validate and expand upon these results.

# Introduction

\*\*Introduction\*\*

Avobenzone is an effective organic UV filter, but its application is limited by significant photodegradation upon UV exposure. Enhancing the photostability of avobenzone is crucial for developing long-lasting sun protection products. This project investigates the encapsulation of avobenzone within biodegradable poly(lactic-co-glycolic acid) (PLGA) microspheres as a strategy to protect the compound from oxidative degradation and improve its stability.

This report details a systematic evaluation of this approach. The investigation began by establishing reliable spectrophotometric methods to quantify avobenzone and a comparative compound, octocrylene, determining their respective absorption maxima at 357 nm and 310 nm. Subsequently, a series of PLGA microsphere formulations (e.g., AV9-AV16) were prepared with varying polymer concentrations and evaluated for microsphere yield and drug loading efficiency. The protective efficacy of the encapsulation was then assessed by comparing the UV-induced degradation rate of encapsulated avobenzone against its unencapsulated form. Finally, drug release studies were conducted on select formulations to characterize the release kinetics of avobenzone from the microspheres over time.

# Objectives

Objectives:

The project had the following objectives:

1. To prepare avobenzone-loaded microspheres with varying concentrations of PLGA and evaluate their drug loading percentages and formulation yields.

2. To measure the degradation of avobenzone and octocrylene, both individually and in combination, after exposure to UV radiation.

3. To evaluate the protective effect of PLGA microsphere encapsulation on the stability of avobenzone under UV exposure.

4. To assess the drug release profiles of avobenzone-loaded PLGA microsphere formulations over designated time points.

# Methodology

Methodology:

## Preparation of Avobenzone-Loaded Microspheres

Avobenzone-loaded microspheres were prepared using various concentrations of poly(lactic-co-glycolide) (PLGA; Viatel DLG 7502E, 75:25 lactide:glycolide). The dispersed phase for each formulation was created by dissolving specified masses of avobenzone and PLGA into dichloromethane (DCM). Multiple formulations were synthesized (designated AV10 through AV16) to achieve increasing polymer concentrations, ranging from 52.5 mg/mL to 65 mg/mL, which corresponded to a 5% to 30% increase over a baseline PLGA concentration of 50 mg/mL. The specific masses of avobenzone and PLGA, along with the volume of DCM used for each formulation, are detailed in [TABLE\_1].

\*\*Table 1: Formulation parameters for avobenzone-loaded PLGA microspheres.\*\*

## Spectrophotometric Characterization of UV Filters

The absorbance characteristics of avobenzone and octocrylene were determined using a Tecan Spectrophotometer. Stock solutions were prepared at a concentration of 1 mg/mL for each compound individually in both dimethyl sulfoxide (DMSO) and ethanol. Additionally, mixture solutions containing 0.5 mg/mL of both avobenzone and octocrylene were prepared in each solvent. These stock solutions were pipetted into a Greiner UV half area 96-well plate and serially diluted. The absorbance of each well was measured across a wavelength range of 230 nm to 425 nm to identify the maximum absorption peaks for each compound and solvent condition as shown in the absorbance spectra for octocrylene in DMSO [FIGURE\_1], octocrylene in ethanol [FIGURE\_2], avobenzone in DMSO [FIGURE\_3], avobenzone in ethanol [FIGURE\_4], the mixture in DMSO [FIGURE\_5], and the mixture in ethanol [FIGURE\_6].

\*\*Figure 1: Absorbance spectrum of octocrylene in DMSO.\*\*

\*\*Figure 2: Absorbance spectrum of octocrylene in ethanol.\*\*

\*\*Figure 3: Absorbance spectrum of avobenzone in DMSO.\*\*

\*\*Figure 4: Absorbance spectrum of avobenzone in ethanol.\*\*

\*\*Figure 5: Absorbance spectrum of an avobenzone and octocrylene mixture in DMSO.\*\*

\*\*Figure 6: Absorbance spectrum of an avobenzone and octocrylene mixture in ethanol.\*\*

## Photodegradation of Avobenzone and Octocrylene Solutions

The photodegradation of avobenzone and octocrylene in solution was evaluated using several UV exposure methods. Standard solutions of 0.1 mg/mL avobenzone, 0.3 mg/mL octocrylene, and a mixture of both in DMSO were prepared. Aliquots of 250 µL were added to the wells of Costar 24-well plates. One set of plates was exposed to a UV source, while a corresponding control plate was stored in a dark area. The UV sources included a 5.0 UVB bulb lamp apparatus and a Formlabs UV Cure Chamber. Samples were exposed for various durations, with timepoints measured from 10 minutes up to 24 hours. Degradation was quantified by measuring the absorbance at 365 nm at each timepoint using a Nanodrop One or OneC Spectrophotometer. The absorbance values for UV-exposed and non-exposed avobenzone, octocrylene, and their mixture are presented in [TABLE\_2], [TABLE\_3], and [TABLE\_4], respectively.

\*\*Table 2: Absorbance at 365 nm for UV-exposed and non-exposed 0.1 mg/mL avobenzone solutions over time.\*\*

\*\*Table 3: Absorbance at 365 nm for UV-exposed and non-exposed 0.3 mg/mL octocrylene solutions over time.\*\*

\*\*Table 4: Absorbance at 365 nm for UV-exposed and non-exposed avobenzone-octocrylene mixture solutions over time.\*\*

## Photoprotective Efficacy of PLGA Microspheres

The ability of PLGA microspheres to protect avobenzone from UV-induced degradation was assessed. Predetermined masses of various avobenzone-loaded microsphere formulations (AV7, AV8, AV9, AV12, AV13, AV14, and AV15), ranging from 2.2 mg to 12.1 mg, were weighed into the wells of Costar 24-well plates [TABLE\_5]. The plates were then subjected to UV radiation from either a UVB bulb or a Formlabs UV Cure Chamber for specified time courses, including intervals of 10 minutes up to a total of 50 minutes. Following UV exposure, the microspheres from each timepoint were recovered from the wells using approximately 2 mL of deionized water and transferred to centrifuge tubes. The samples were centrifuged at 15,000 rcf for 10 minutes to pellet the microspheres. The aqueous supernatant was decanted, and the pellet was dissolved in DMSO. These solutions were further diluted, typically in a 1:10 ratio with DMSO, to achieve a final concentration suitable for spectrophotometric analysis. The extent of avobenzone degradation was determined by measuring the absorbance at 365 nm using a Nanodrop or Tecan spectrophotometer, with results summarized in [TABLE\_6]. The degradation profile over time is illustrated in [FIGURE\_7].

\*\*Table 5: Experimental parameters for avobenzone microsphere degradation studies, including formulation, mass, and PLGA concentration.\*\*

\*\*Table 6: Avobenzone content in microsphere formulations at discrete timepoints following UV exposure.\*\*

\*\*Figure 7: Degradation profile of avobenzone in microspheres with increased PLGA concentration after UV exposure.\*\*

# Results

\*\*Results\*\*

## Spectroscopic Characterization of Avobenzone and Octocrylene

The maximum UV-Visible light absorption peaks for avobenzone and octocrylene were determined in both dimethyl sulfoxide (DMSO) and ethanol solvents. Solutions of each compound, as well as a 1:1 mixture, were prepared and serially diluted. The absorbance of each dilution was measured across a wavelength range of 230 nm to 425 nm.

For octocrylene, the maximum absorption peak (λmax) was identified at 310 nm in both DMSO [FIGURE\_1] and ethanol [FIGURE\_2]. For avobenzone, the λmax was observed at 357 nm in both DMSO [FIGURE\_3] and ethanol [FIGURE\_4]. When combined in a 1:1 mixture, the resulting spectra in both DMSO [FIGURE\_5] and ethanol [FIGURE\_6] displayed both characteristic peaks, with the avobenzone peak at 357 nm being more prominent. A partial overlap was observed between the decreasing shoulder of the octocrylene peak and the increasing shoulder of the avobenzone peak.

\*\*Figure 1: Absorbance spectrum of octocrylene in DMSO.\*\*

\*\*Figure 2: Absorbance spectrum of octocrylene in ethanol.\*\*

\*\*Figure 3: Absorbance spectrum of avobenzone in DMSO.\*\*

\*\*Figure 4: Absorbance spectrum of avobenzone in ethanol.\*\*

\*\*Figure 5: Absorbance spectrum of a 1:1 mixture of avobenzone and octocrylene in DMSO.\*\*

\*\*Figure 6: Absorbance spectrum of a 1:1 mixture of avobenzone and octocrylene in ethanol.\*\*

## Formulation and Characterization of Avobenzone-Loaded PLGA Microspheres

Avobenzone-loaded microspheres were prepared using PLGA concentrations ranging from 52.5 mg/mL to 65 mg/mL, corresponding to +5%, +10%, +20%, and +30% increases over a baseline concentration. The resulting microsphere yield, percent yield, and drug loading were quantified for each formulation [TABLE\_1].

Initial formulations AV10 (+5% PLGA) and AV11 (+10% PLGA) produced low microsphere yields of 3.0 mg (2.91% yield) and 18.0 mg (17.03% yield), respectively. These were reformulated as AV14 and AV15, which produced higher yields. Across the successful formulations (AV12, AV13, AV14, AV15, AV16), microsphere yields ranged from 21.4 mg to 47.8 mg. The measured drug loading percentage (w/w) was consistent across formulations, ranging from 41.39% to 48.79%.

\*\*Table 1: Yield and drug loading characteristics of avobenzone-loaded PLGA microsphere formulations.\*\*

| Formulation Code | PLGA Concentration Increase | Microsphere Yield (mg) | Percent Yield | Drug Loading % (w/w) |

| :--- | :--- | :--- | :--- | :--- |

| AV10 | +5% | 3.0 | 2.91% | 48.37% |

| AV11 | +10% | 18.0 | 17.03% | 47.02% |

| AV12 | +20% | 26.9 | 24.34% | 41.39% |

| AV13 | +30% | 43.2 | 37.31% | 42.71% |

| AV14 | +5% | 21.4 | 20.70% | 43.02% |

| AV15 | +10% | 47.8 | 45.22% | 41.93% |

| AV16 | +20% | 40.1 | 36.33% | 48.79% |

## In Vitro Release of Avobenzone from Microspheres

The in vitro release of avobenzone from microsphere formulations AV10 and AV15 was monitored over a 56-hour period. The cumulative percentage of released drug was calculated at multiple timepoints [FIGURE\_7]. Formulation AV10 exhibited an initial burst release of 12% at time 0, reaching 58% cumulative release by 8 hours and 113% by 56 hours. Formulation AV15 showed a similar initial burst of 14% at time 0, followed by a more gradual release, reaching 28% by 8 hours and a total cumulative release of 74% at the 56-hour timepoint.

\*\*Figure 7: Cumulative percentage of avobenzone released from AV10 and AV15 microsphere formulations over 56 hours.\*\*

## Photodegradation of Avobenzone

The photodegradation of unencapsulated avobenzone and octocrylene in DMSO solution was evaluated following exposure to a UV cure chamber. Absorbance was measured at 365 nm at various timepoints.

In one study, a 0.1 mg/mL solution of avobenzone was exposed for up to 150 minutes. The absorbance at 365 nm decreased from an initial value of 11.776 to a minimum of 1.213 after 60 minutes, representing an 89.7% reduction [TABLE\_2]. For exposure times beyond 60 minutes, the absorbance value increased, reaching 3.679 at 150 minutes. A separate experiment with an 80-minute exposure showed a maximal absorbance decrease of 70.2% at 20 minutes (from 12.0870 to 3.6020), followed by fluctuating absorbance values at later timepoints.

In contrast, a 0.3 mg/mL solution of octocrylene showed no significant degradation. The absorbance at 365 nm remained stable, measuring 0.7290 at time 0 and 0.7850 after 80 minutes of UV exposure [TABLE\_2]. A 1:3 mixture of avobenzone (0.1 mg/mL) and octocrylene (0.3 mg/mL) showed a 75.4% decrease in absorbance after 50 minutes of UV exposure (from 1.3320 to 0.3280). The degradation profiles of UV-exposed and non-exposed (control) samples are presented graphically [FIGURE\_8, FIGURE\_9].

\*\*Table 2: Absorbance at 365 nm for free avobenzone, octocrylene, and a mixture at key UV exposure timepoints.\*\*

| Sample | Concentration | Initial Absorbance (T=0) | Time of Max Degradation (min) | Absorbance at Max Degradation | % Decrease |

| :--- | :--- | :--- | :--- | :--- | :--- |

| Avobenzone | 0.1 mg/mL | 11.776 | 60 | 1.213 | 89.7% |

| Avobenzone | 0.1 mg/mL | 12.087 | 20 | 3.602 | 70.2% |

| Octocrylene | 0.3 mg/mL | 0.729 | 80 | 0.785 | None |

| Avo/Octo Mix | 0.1 / 0.3 mg/mL | 1.332 | 50 | 0.328 | 75.4% |

\*\*Figure 8: Absorbance at 365 nm over 80 minutes for UV-exposed and non-exposed (control) solutions of avobenzone, octocrylene, and an avobenzone-octocrylene mixture.\*\*

\*\*Figure 9: Absorbance at 365 nm over 80 minutes for UV-exposed avobenzone and avobenzone-octocrylene mixture solutions.\*\*

The ability of PLGA microspheres to protect encapsulated avobenzone from photodegradation was assessed. Four formulations with increasing PLGA content—AV14 (+5%), AV15 (+10%), AV12 (+20%), and AV13 (+30%)—were exposed to UV radiation for up to 50 minutes. The absorbance at 365 nm was measured at 10-minute intervals [TABLE\_3].

The formulations with +10% (AV15) and +20% (AV12) PLGA concentrations maintained higher relative absorbance values through the first 20 minutes of UV exposure compared to the +5% (AV14) and +30% (AV13) formulations [FIGURE\_10]. The AV15 formulation showed an initial absorbance of 1.889, which remained at 1.935 after 20 minutes. The AV12 formulation began at 1.189 and measured 1.167 at 20 minutes. In contrast, the AV14 formulation's absorbance decreased from 1.978 to 1.551, and the AV13 formulation's absorbance decreased from 2.175 to 0.226 over the same period. After 20 minutes, all formulations exhibited varied degradation patterns.

\*\*Table 3: Absorbance at 365 nm for avobenzone-loaded microspheres with increasing PLGA concentrations during UV exposure.\*\*

| Timepoint (Minutes) | AV14 (+5% PLGA) | AV15 (+10% PLGA) | AV12 (+20% PLGA) | AV13 (+30% PLGA) |

| :--- | :--- | :--- | :--- | :--- |

| 0 | 1.978 | 1.889 | 1.189 | 2.175 |

| 10 | 1.640 | 1.991 | 1.041 | 1.112 |

| 20 | 1.551 | 1.935 | 1.167 | 0.226 |

| 30 | 2.044 | 1.134 | 0.316 | 1.658 |

| 40 | 1.590 | 2.406 | 0.759 | 2.423 |

| 50 | 1.631 | 1.624 | 0.045 | 3.478 |

\*\*Figure 10: Absorbance at 365 nm versus UV exposure time for avobenzone-loaded microspheres with varied PLGA concentrations.\*\*

\*\*Most Impactful Features:\*\*

\* \*\*PLGA Concentration (+10% and +20%)\*\* — Enhanced photostability. Formulations AV15 and AV12 showed minimal to no decrease in avobenzone absorbance after 20 minutes of UV exposure, suggesting that these intermediate polymer concentrations provide superior protection against initial degradation compared to lower (+5%) or higher (+30%) concentrations.

# Conclusion

Encapsulation of avobenzone in poly(lactic-co-glycolic acid) (PLGA) microspheres is a viable strategy for mitigating UV-induced photodegradation. Spectroscopic characterization established maximum absorption peaks for avobenzone and the photostabilizer octocrylene at 357 nm and 310 nm, respectively. Degradation studies confirmed that octocrylene is photostable, while unencapsulated avobenzone degrades rapidly, with peak degradation occurring within 20 to 60 minutes of high-intensity UV exposure.

The protective efficacy of the PLGA microspheres is highly dependent on polymer concentration. Formulations with 10% (AV15) and 20% (AV12) PLGA content provided significant protection, delaying degradation for up to 20 minutes. In contrast, formulations with lower (+5% PLGA, AV14) or higher (+30% PLGA, AV13) polymer content exhibited immediate degradation. A separate 56-hour study confirmed that encapsulated avobenzone is released from the microspheres over time. Although complete photostabilization was not achieved, these findings demonstrate that optimizing PLGA concentration is critical for enhancing the photostability of avobenzone through microencapsulation.